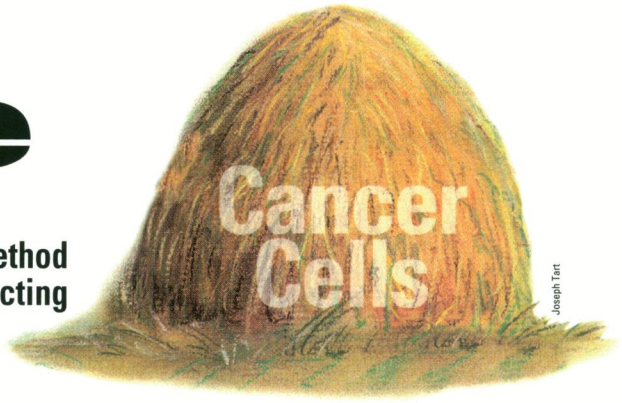
Finding the Needle in a Haystack: New Method for Detecting Cancer Cells



Cancer has touched or transformed the lives of millions of people. The American Cancer Society estimates that there are about 1.2 million new cases of cancer per year. Environmental factors, such as chemicals, radiation, and viruses, as well as internal factors such as hormones and inherited mutations, may all contribute to development of various cancers. Successful diagnosis and treatment of cancers relies in part on detecting cancer cells within the body. Recently, a team of researchers led by Hans-Joachim Gross at the M.D. Anderson Cancer Center developed a new technique to detect very low levels of cancer cells in clinical samples of normal cells in blood and bone marrow. This innovative technique uses flow cytometry and allows researchers to find 1 cancer cell in 10 million normal cells, a detection level about 20 times more sensitive than is possible with current methods such as immunocytochemistry and polymerase chain reaction.

Applications

Current techniques for detecting low levels of cancer cells can provide different types of information for researchers and clinicians. For example, a blood sample from a patient that is newly diagnosed with cancer can be tested to determine if the cancer is spreading to other areas. Patient blood samples can also be tested after treatment with chemotherapy to determine if the number of tumor cells has decreased.

Another application of the detection methods is for testing samples of bone marrow that are removed from cancer patients undergoing bone marrow transplants. Bone marrow transplants are currently used to treat a variety of blood cancers including leukemia, lymphomas, and multiple myelomas, many of which may be environmentally mediated. Marrow transplants may also be used as part of a course of therapy for nonhematologic diseases such as solid tumors, immunological dysfunctions, and inherited enzyme deficiencies. Gross's new flow cytometry assay is primarily targeted for application as a cancer treatment. In addition to being a more sensitive cancer cell detection method, the new flow cytometry assay is unique in that it may someday be used to remove cancer cells from a sample of bone marrow. Although some years away from practical use, this process may eventually decrease the likelihood of a cancer recurrence after the bone marrow transplant.

Flow Cytometry

Flow cytometry is based on analysis of light scatter and staining from a continuous sample of cells as they pass a detector. The light source is typically one or more lasers. The detectors capture forward and side scatter of light as each cell flows through the analysis point. This highly sensitive technique allows the characteristics of cells or other particles to be studied in great detail.

Lasers are a good light source because they can provide a bright, narrow, stable beam. However, the color output of laser light is very restricted. This disadvantage restricts the choices of dyes that can be visualized in a flow cytometer. Increasing the number of lasers allows additional colors to be visualized and increases the sensitivity of the analysis. The flow cytometer that Gross uses in the detection step contains three lasers in a complex arrangement that allows analysis of a large number of experimental parameters, thereby increasing the accuracy of the data.

In addition to measuring light scatter, some flow cytometers can sort cells based on the signal they emit. Cells can be stained with antibodies linked to colored dyes. Then, as the sample is run through the sorter, all cells emitting a specific color light signal (e.g., red) will be physically shunted into a collection test tube, while cells with other colors (e.g., green) will be collected in a second tube. In his model system, Gross used a FACS (fluorescence-activated cell sorter) to sort BT-20 breast carcinoma cells. The BT-20 cells were labeled with a UV-excitable blue dye called 7-amino-4-chloromethylcoumarin (CMAC). This dye allowed the detector to count the "blue" cells and deposit either 0, 40, 400, or 4,000 cells into four tubes each containing approximately 1×10^8 peripheral blood stem cells. The purpose was to create experimental samples with a known number of cancer cells. The mock samples were run through a detector, and the number of cancer cells actually detected was matched against the number that should have been detected.

A second flow cytometer with three lasers was used for the detection step. Researchers stained the BT-20 cancer cells to distinguish them from the stem cells. Several different antibodies against each cell type were used to label the cell mixture to ensure the most accurate detection. The rare cancer cells were stained with three antibodies linked to dyes that are yellow and two shades of red. The stem cells were stained with a panel of five antibodies all linked to a green dye called fluorescein isothiocyanate. In his study, published in the January issue of the *Proceedings of the National Academy of Sciences*, Gross said, "The requirement that the rare cancer cell be simultaneously positive for three separate colors and negative for a fourth color [allows] detection of as few as one cancer cell in 10^7."

The signal from the cell samples was then transmitted to a computer which generates a scatter plot of the BT-20 cells. The blue CMAC dye originally used to count the cancer cells was then used as a final test to validate the results from the

selective sorting of cancer and stem cells. After the red and yellow cells were plotted, these cells were examined under a UV light. The presence of blue UV sensitive dye in all of these cells helped confirm that the assay was successful in sorting the cancer cells.

Better Marrow

Bone marrow is usually collected for an autologous transplant when a patient is in remission or early in the disease, but there is a chance that cancer cells may also be collected. In order to return marrow that is as cancer-free as possible, some bone marrow transplant centers purge the marrow. One common technique for purging uses a cytotoxic agent to selectively damage cancer cells, but unfortunately it does not completely spare the undiseased marrow cells. Another technique, "negative selection," couples antibodies that recognize and capture specific cancer cell marker proteins to a solid matrix in a column or to magnetic beads. The sample then is mixed with the beads or run over the column and the cancer cells are captured and removed. A third, promising technique uses "positive selection" to capture stem cells and to remove cancer cells. This procedure uses an antibody column that binds to a $CD34^+$ marker on stem cells.

"In model studies," writes Gross, "bone marrow with 10% tumor cell contamination can be purged by a factor of 10^{-3} to 10^{-4}. In a more realistic example with 0.1% tumor cell contamination, about 3,000 tumor cells could be returned into the patient. To detect those cells in purged bone marrow before infusion requires a detection level of one tumor cell per 10^6 to 10^7 cells, a level not achieved so far. Present day methodologies . . . permit a level of detection by microscopy of one tumor cell per 10^5 cells at best."

Gross asserts that one major application for his technique is marrow sample testing to improve diagnostic capability. Unfortunately, a major roadblock for using this process is the time needed to analyze an entire marrow sample of 100 million cells. As a sample of cells drips through the laser, about 6,000 "events" (light scatter from a cell) per second are recorded by the detectors. It may take up to seven hours to analyze a typical sample, which is not a clinically useful timeframe.

To address this concern, a company in Palo Alto, California called SyStemix has developed a high-speed flow cytometry system designed to sort bone marrow cells. SyStemix, which explores stem cell biology and therapy, has developed a cytometer that can analyze about 50,000 events per second. In combination with a positive selection column that reduces the total number of cells in the sample to be sorted, this system reduces the sort time of a typical sample to about one to two hours. There is, however, a tradeoff between reduced analysis time and the possibility of increased stem cell damage that can result from manipulation. Other laboratory technology companies are also working to increase efficiency and decrease cost of purging methods.

Even though removal of cancer cells from reinfused marrow seems logical, purging is a controversial topic among researchers and clinicians. There is no solid evidence to support whether a relapse is caused by tumor cells in reinfused marrow or by original tumor cells that survive the ablation process. Many studies of autologous transplant patients have shown no difference in survival between patients that receive purged marrow versus those that receive nonpurged marrow.

A prospective clinical trial conducted by the European Bone Marrow Transplant registry is attempting to provide data to support or refute the benefits of marrow purging. All trial centers are testing a standardized antibody-linked magnetic bead technique. The results will not be available for years because a large number of patients must be recruited to complete the study.

Meanwhile, cancer patients who receive bone marrow transplants stand to benefit from improved procedures that will augment their quality of life and increase their life expectancy. Improved detection techniques will will also create new opportunities to research and study many types of cancer in hope of a cure.

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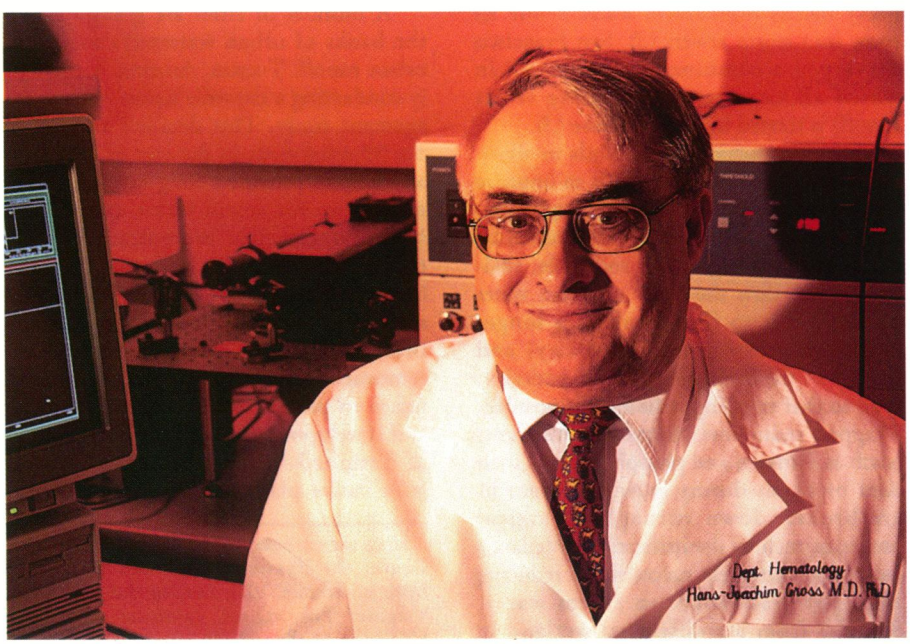

F. Carter Smith

Cell sleuth. Hans-Joachim Gross and colleagues at the M.D. Anderson Cancer Center have developed a cancer detection method that is one-in-ten-million.

Suggested Reading

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